REMARKS

The Official Action dated May 7, 2002 and the Advisory Action dated September 9, 2002 have been carefully considered. Additionally, the telephone interview which the Examiner courteously afforded Applicants' representatives is acknowledged and appreciated. Accordingly, the changes presented herewith, taken with the following remarks, are believed sufficient to place the present application in condition for allowance. Reconsideration is respectfully requested.

Although, a formal agreement regarding the claims was not reached during the aforementioned interview, the claim amendments presented herein were discussed and are believed to overcome the outstanding rejections.

More particularly, the specification has been amended, in accordance with the Advisory Action, to clarify that SPI-GHRE, SEQ ID NO:1, is 52 bp. Claims 1 and 19 are amended to recite the transitional phrase "comprising" which was inadvertently omitted in the previous amendment of the claims. Claims 1 and 44 are amended to clarify that the promoter is "operably linked" to the structural gene. Claims 8 and 10 are amended to clarify the limitations therein in accordance with the teaching of the specification at page 3, lines 1-12, page 4, and original claims 1, 3 and 4. Claims 5, 15 and 23 are amended to clarify that the DNA construct is "incorporated" into a genome of a eukaryotic cell, in accordance with the teachings of the specification. Claim 19 is further amended to clarify the method step of "incorporating" the nucleotide sequence consisting of TTCTGAGAA "into the first DNA construct" upstream of the promoter. Claims 27 and 30 are amended to clarify the arrangement of the recited elements. A Version With Markings Showing Changes Made is attached. It is believed that these changes do not involve any introduction of new matter, and do not raise any new issues subsequent to final rejection of the claims whereby entry is believed to be in order and is respectfully requested.

Claims 8-11, 16, 17 and 27-32 were rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. With respect to claims 8-11, 16 and 17, the Examiner asserted that the specification does not contemplate using any enhancer element other than those comprising a nucleotide sequence TTCTGAGAA. With respect to claims 27-32, the Examiner asserted that the specification does not contemplate or describe expression factors or DNA constructs comprising an enhancer element incorporated within a structural gene.

This rejection is traversed with respect to present claims 8-11, 16, 17 and 27-32, and reconsideration is respectfully requested. More particularly, claim 8 is directed to an expression vector. The expression vector comprises a structural gene encoding a desired protein or polypeptide and a promoter. The vector further comprises six enhancer elements and each of the enhancer elements consists essentially of the nucleotide sequence SEQ ID NO:1. Claim 10 is directed to an expression vector. The expression vector comprises a structural gene encoding a desired protein or polypeptide and a promoter. The vector further comprises six enhancer elements and each of the enhancer elements consists essentially of the nucleotide sequence TTCTGAGAA. As the Examiner noted in the Official Action and the Advisory Action, the specification discloses an enhancer element comprising the nucleotide sequence TTC TGA GAA, for example, at page 5, original claims 1 and 3. Moreover, original claim 4 further describes the enhancer element as comprising single or multimeric copies of SPI-GHRE or a derivative thereof, while the specification discloses at page 3, lines 5-7 six repeats of SPI-GHRE and at lines 9-10 six repeats of SPI-GAS, identified as the core sequence (page 3, lines 1-2). Thus, the specification, including the original claims, fully describe the expression vectors of claim 8 and 10.

Claim 27 is directed to an expression vector. The expression vector comprises a structural gene encoding a protein, a promoter upstream of and operably linked to the structural gene, and at least one enhancer element consisting essentially of a nucleotide sequence TTCTGAGAA upstream or the promoter. Finally, claim 30 is directed to a DNA construct. The DNA construct comprises a structural gene encoding a protein, a protein upstream of and operably linked to the structural gene, and at least one enhancer element consisting essentially of a nucleotide sequence TTCTGAGAA upstream of the promoter. The specification discloses such expression vector and DNA construct, for example, at page 3. The phrase "incorporated within the structural gene" has been omitted from these claims. Thus, claims 27 and 30 are fully described in the specification.

It is therefore submitted that present claims 8-11, 16, 17 and 27-32 are fully described in the specification and the rejection under 35 U.S.C. §112, first paragraph, has been overcome. Reconsideration is respectfully requested.

Claims 1, 2, 5, 7, 10, 15, 19-21, 23-27, 30, 39, 40, 44, 45, 49, 52 and 53 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite. With respect to claims 1, 2, 39, 40, 51 and 52, the Examiner asserted that the claim language does not indicate whether the method "comprises" or "consist of" the method steps. Accordingly, claim 1 has been amended to clarify that the method "comprises" the method steps. Claim 19, although not specified by the Examiner in this rejection, has also been so amended.

With respect to claims 5, 7, 15, 23-26 and 53, the Examiner asserted that the recitation of "a DNA construct transfected into the genome of a eukaryotic host cell" is indefinite because a DNA construct is transfected into a cell, not into a genome. Accordingly, claims 5, 15 and 23 have been amended to clarify that the DNA construct is "incorporated" into a genome of a eukaryotic cell.

With respect to claim 10, the Examiner asserted that the recitation of "the enhancer element" is ambiguous because the claim recites six enhancer elements and each can be different from all the others, and therefore, it is unclear to which enhancer element the claim is referring. Accordingly, claim 10 has been amended to recite "at least one of" the enhancer elements.

With respect to claims 19-21 and 49, the Examiner asserted that "providing" is not sufficient to incorporate the enhancer element into the DNA construct upstream of the promoter. The Examiner further asserted that the recitation of "the DNA construct" in step (c) and (d) and at the end of claim 19 is indefinite because the phrase has ambiguous antecedent basis. Accordingly, claim 19 has been amended to clarify the limitations therein.

With respect to claims 27 and 30, the Examiner asserted that the recitation of "wherein the enhancer element is incorporated within the structural gene by transfection" is indefinite because cells, not genes, are transfected. The Examiner further asserted that incorporation of the enhancer element within the structural gene would disrupt the coding sequence. Claims 27 and 30 have been amended to clarify the arrangement of the elements of the expression vector of claim 27 and the DNA construct of claim 30, and to omit the recitation of incorporation within the structural gene.

With respect to claims 44 and 45, the Examiner asserted that it is unclear whether the promoter is operably linked to the structural gene. Claim 44 has been amended to recite that "the promoter is operably linked to the structural gene".

It is therefore submitted that claims 1, 2, 5, 7, 10, 15, 19-21, 23-27, 30, 39, 40, 44, 45, 49, 52 and 53, are definite and the rejection under 35 U.S.C. §112, second paragraph, has been overcome. Reconsideration is respectfully requested.

Claims 27-32 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lindquester et al (1989). The Examiner asserted that Lindquester et al need not disclose the function of the sequence because the claims are directed to compositions that include the sequence.

However, as discussed during the interview and as set forth in detail below, Applicants submit that the respective expression vector and DNA construct defined by claims 27-32 are nonobvious over and patentably distinguishable from the teachings of Lindquester et al. Accordingly, this rejection is traverse and reconsideration is respectfully requested.

More specifically, claim 27 is directed to an expression vector comprising a structural gene encoding a protein, a promoter upstream of and operable linked to the structural gene, and at least one enhancer element consisting essentially of a nucleotide sequence TTCTGAGAA upstream of the promoter. Claim 30 is directed to a DNA construct comprising a structural gene encoding a protein, a promoter upstream of and operably linked to the structural gene, and at least one enhancer element consisting essentially of a nucleotide sequence TTCTGAGAA upstream of the promoter.

In contrast, Lindquester et al describe their study of avian tropomyosin gene expression. As noted by the Examiner, Lindquester et al disclose that avian tropomyosin gene includes sequence TTCTGAGAA located in one of the introns, specifically at position 18602 of Figure 1 on page 2105, and that a genomic clone containing tropomyosin gene was isolated from a quail DNA genomic library.

However, Applicants find no teaching or suggestion by Lindquester et al of an expression vector as defined in claim 27 or a DNA construct as defined in claim 30 comprising, inter alia, a structural gene encoding a protein, a promoter upstream and operably linked to a structural gene, and at least one enhancer element consisting essentially of a

nucleotide sequence TTCTGAGAA upstream of the promoter. Moreover, the mere teaching by Lindquester et al of the avian tropomyosin gene including the nucleotide sequence TTCTGAGAA, without further teaching, suggestion or recognition of the ability of the nucleotide sequence to act as an enhancer element, provides no teaching or suggestion to one of ordinary skill in the art to produce either an expression vector or a DNA construct as recited in claims 27 and 30.

References relied upon to support a rejection under 35 U.S.C. §103 must provide an enabling disclosure, i.e., they must place the claimed invention in the possession of the public, *In re Payne*, 203 U.S.P.Q. 245 (CCPA 1979). In view of the failure of Lindquester et al to teach or suggest the expression vector of claim 27 or the DNA construct of claim 30, the reference does not provide an enabling disclosure of the present invention, and therefore, does not support a rejection of the claims under 35 U.S.C. §103. It is therefore submitted that the rejection of claims 27-32 under 35 U.S.C. §103 has been overcome. Reconsideration is respectfully requested.

It is believed that the above represents a complete response to the Examiner's rejections under 35 U.S.C. §§ 103 and 112, first and second paragraph, and places the present application in condition for allowance. Reconsideration and an early allowance are requested. In the event that the present application is not in condition for allowance, entry of the present amendment for purposes of appeal is requested.

Respectfully submitted,

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VERSION WITH MARKINGS SHOWING CHANGES MADE

In the Specificati n:

The last paragraph on page 2 to the last paragraph on page 3 is amended as follows:

Example 1. Identification of a core GH regulated sequence.

The [50] 52 bp SPI-GHRE;

(GATCTACGCTTCTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCCATG) was used to identify a core GH regulated sequence using gel electrophoresis mobility shift assay (GEMSA). Nuclear extracts were prepared and incubated with a 32P labeled [50] 52 bp SPI-GHRE. Subsequently the extracts were analysed on polyacrylamide gels. The results showed that nuclear proteins, dependent on GH, bound to this DNA sequence. By competition with shorter oligonucleotides derived from SPI-GHRE a core GH sequence was identified. Based on certain sequence homologies to interferon response-elements we called this sequence SPI-GAS and also demonstrated that SPI-GAS functions as a GH regulated DNA element when put into a reporter vector. The core SPI-GAS has the following sequence; TTCTGAGAA.

Example 2. Prolactin and growth hormone both activate SPI-TK reporter gene.

An expression plasmid containing a recombinant hormone responsive reporter consisting of six repeats of a [50] 52 bp growth hormone responsive element (GH-RE) from the serine protease inhibitor (SPI) 2.1 promoter fused to the thymidine kinase (TK) promoter was constructed. Corresponding constructs were made using the SPI-GAS element. Variants expressing either the bacterial protein chloramphenicol acetyl transferase (CAT) or firefly luciferase (SPI-CAT or SPI-Luc respectively) cDNAs were then constructed. Techniques to make these vectors are well known to experts in the field. The plasmid DNA constructions were transfected, together with plasmid expression vectors encoding either rat growth hormone receptors or mouse prolactin receptors, into Chinese hamster ovary (CHO), COS, and Buffalo rat liver (BRL) cells, using DOTAP liposomes and according to the manufacturer

instructions. Cells were incubated overnight with DNA and DOTAP in serum free media, left and then exposed to growth hormone or prolactin for 12 hours. Cell lysates were then prepared and CAT or luciferase enzyme activity measured. Both growth hormone and prolactin treatment lead to an approximately 5-fold stimulation reporter enzyme expression relative to transfected but non-hormone treated cells. These results show that both growth hormone and prolactin can regulate the reporter construct and that a requisite for this is the presence of SPI elements. The core element in the SPI-TK-reporter gene that confers GH regulation is likely to be; TTCTGAGAA, and similar results can be obtained with this element termed SPI-GLE as with the longer, [50] 52 bp element named SPI-GHRE.

Example 3. Multimeric SPI elements in front of a TK promoter give a better response.

Reporters plasmids containing one to six copies of the [50] 52 bp SPI element fused to the TK promoter were constructed. The growth hormone responsiveness of these constructs was tested by transfection into a CHO cell line that stably expresses the rat growth hormone receptor DNA. Growth hormone stimulation of these cells showed that multimerization of SPI elements resulted in a larger growth hormone response.

In the Claims:

Claims 1, 5, 8, 10, 15, 19, 23, 27, 30 and 44 are amended as follows:

1. (Seventh Amendment) An in vitro method of enhancing the transcription of a gene in a DNA construct when the DNA construct is incorporated into the genome of a eukaryotic host cell, the method comprising:

(a) providing a DNA construct comprising a structural gene for a desired protein or polypeptide, a gene promoter upstream of and operably linked to the structural gene, and six copies of an enhancer element upstream of the promoter;

(b) transfecting the eukaryotic host cell to incorporate the DNA construct into the genome of the host cell; and

(c) exposing the DNA construct in the eukaryotic cell to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof;

wherein the enhancer element comprises the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence does not contain the DNA sequence of nucleotide sequence SEQ ID NO:1, and wherein the enhancer element is responsive to both lactogenic hormones and somatogenic hormones.

5. (Sixth Amendment) An enhancer element which when used in a DNA construct for transfection of a eukaryotic host cell is responsive to hormonal stimuli, said enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA, wherein the cnhancer element is responsive to both lactogenic hormones and somatogenic hormones when used in a DNA construct [transfected] incorporated into the genome of a eukaryotic host cell.

8. (Fifth Amendment) An expression vector comprising a structural gene encoding a desired protein or polypeptide and a promoter, wherein the vector further comprises six enhancer elements, and further wherein [at least one of] each of the enhancer elements consists essentially of the nucleotide sequence [TTCTGAGAA] SEQ ID NO:1.

10. (Sixth Amendment) [The expression vector according to claim 9,] An expression vector comprising a structural gene encoding a desired protein or polypeptide and a promoter, wherein the vector further comprises six enhancer elements, and further wherein each of the enhancer elements consists essentially of [element comprises at least one copy of] the nucleotide sequence [SEQ ID NO:1] TTCTGAGAA.

15. (Third Amendment) The enhancer element of claim 5, wherein the enhancer element is responsive to signals generated from both growth hormone and prolactin receptors when used in a DNA construct [transfected] incorporated into the genome of a eukaryotic host cell.

19. (Fifth Amendment) An in vitro method of enhancing the transcription of a gene in a DNA construct, the method comprising:

(a) providing a first DNA construct comprising a structural gene and a promoter upstream of the structural gene,

(b) [providing] incorporating [the DNA construct with] the nucleotide sequence consisting of TTCTGAGAA into the first DNA construct upstream of the promoter, thereby producing a second DNA construct;

(c) transfecting a eukaryotic host cell to incorporate the second DNA construct into the genome of the host cell; and

(d) exposing the second DNA construct in the eukaryotic host cell to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof.

23. (Fourth Amendment) An enhancer element responsive to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof when the enhancer element is [used] in a DNA construct [for transfection] incorporated into a genome of a eukaryotic host cell; wherein the enhancer element consists essentially of the nucleotide sequence TTCTGAGAA.

27. (Fifth Amendment) An expression vector comprising a structural gene encoding a protein, a promoter upstream of and operably linked to the structural gene, and at

least one enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA upstream of the promoter[, wherein the enhancer element is incorporated within the structural gene by transfection].

30. (Fourth Amendment) A DNA construct comprising a structural gene encoding a protein, a promoter upstream of and operably linked to the structural gene, and at least one enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA upstream of the promoter[, wherein the enhancer element is incorporated within the structural gene by transfection].

44. (Fourth Amendment) An isolated DNA construct comprising a promoter operably linked to [,] a structural gene downstream from said promoter, and six repeats of an enhancer element upstream from said promoter, wherein the enhancer element consists essentially of the sequence TTCTGAGAA.